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POTENTIALITY OF *Quercus robur*, L. FOR INDUCING SHOOTLETS MASS PRODUCTION THROUGH TISSUE CULTURE TECHNIQUES AND FACTORS INFLUENCING ITS SUCCESS

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ABSTRACT

This work was investigated in order to obtain mass production of shootlets from *Q. robur*, as a promising tree. Phenol exudates was a major problem, which was overcome by manipulating the mother seedling by a dark system followed by handling the explants obtained by successive steps. The effect of explant position on mother seedling branch, medium type, plant growth regulators (type and concentration), sucrose concentration, and the solidifying agent (agar and gerlite), were investigated. Favourable culture establishment was obtained by inoculating the medial nodes segments on WPM supplemented with 0.2 mg L^{-1} IBA + 0.8 mg L^{-1} BA rather than on QL medium. A high frequency of shootlets multiplication (60/explant) was obtained on WPM supplemented with 3% sucrose and 0.2 mg L^{-1} IBA + 1.2 mg L^{-1} BA. Successful rooting of shootlets was induced by modifying WPM with 0.5 mg L^{-1} IBA and solidified by 0.2% gerlite which surpassed 0.7% agar in rooting criteria.

Keywords: *Quercus robur*, tissue culture, phenol exudates, explant position, gelling agent, mother plant.

INTRODUCTION

English oak, or pedunculate oak (*Quercus robur* L.) is an important forest species (Family Fagaceae), deciduous with monoecious flowers. Outstanding trees can reach 40 meters height and 1 meter diameter, as they grow quite rapidly. Its wood is strong, tough, hard and durable, so it can be used for many purposes, specially ship building, furniture, veneer, parquets and sleepers. Besides, its wood is a very good fuel and its bark is used for tanning (Bailey, 1933; Bellarosa, 1989; James *et al.*, 1994 and Manzanera *et al.*, 1996).

Oak is propagated by acorns sown immediately after collecting in fall. Good acorns harvests are irregular, and long term storage of acorns still presents unsolved problems, as they are considered "recalcitrant seeds" (Guthke and Spethmann, 1993). As well, the long reproductive cycle of oak is a serious obstacle to effective tree improvement by conventional tree breeding techniques. Nevertheless, the vegetative propagation of oak has been considered difficult and has not been successful on a commercial scale. Trees of 20-30 years old produce 11-18% rooted cuttings only (Chalupa, 1993).

In vitro culture techniques may solve most of these problems and conservation of the superior characters of the tree will be possible, as well. Colonal propagation of a selected strain is a useful method of accelerating the improvement of this tree. However, micropropagation of woody and other

species can be much more difficult and labor-intensive if inhibitory exudates are produced. It has been proved that Fagaceae trees, such as *Quercus spp.* are difficult to be cultured *in vitro* (Cai et al., 1987; Preece and Compton, 1991 and Shoyama *et al.*, 1992). This may be due to their tannin production. Nevertheless, some success has been achieved in tissue culture propagation of some *Quercus spp.* such as *Quercus rubra* (Seckinger *et al.*, 1979), *Q. suber* (Pardos, 1981) and *Q. acutissima* (Shoyama *et al.*, 1992). Preece and Compton (1991) obtained the greatest success to overcome the discoloration problem with rapid transfers within the same vessel or to a fresh medium, or with explant pre-soaks up to 4 hours. Similar results were reported by Mervat (1997) on *Ceratonia siliqua* and Hanafy Ahmed *et al.* (2002 b) on *Myrtus communis*. Many methods have been proposed to resolve this problem. Reynolds and Murashige (1979) treated tissues of palm with an antioxidant solution containing 100 mg L^{-1} of ascorbic acid and 150 mg L^{-1} of citric acid. Other researchers used activated charcoal or polyvinylpyrrolidon (PVP), also Nkanka (1982) used vitamin E as antioxidant agent in cultures of shoot tips and nodes of *Q. borealis*.

The objectives of the present work were to elaborate a protocol for a colonal propagation and mass production and to find out a technique to overcome the accompanied micropropagation problems to rescue such threaten germplasm. Spreading out this valuable tree into the relevant locations in Egypt is in correspondence to the germplasm collection and conservation program of the Horticulture Research Institute (H.R.I.).

MATERIALS AND METHODS

I- Donor plants and initial explants:

Nodal segments from three positions (shoot tip, medial 4 node segments and basal 4 node segments) were obtained from about two years old seedlings produced from acorns sown in the greenhouse of Plant Biotechnology Lab., Fac. Agric., Cairo Univ., Giza, as well as the *in vitro* work was adopted in the same Lab. Acorns were obtained from an adult tree (about 38 years old) located at Kanater Experimental Station, Horticulture Research Institute (H.R.I.) and this work was conducted during the successive years of 1999, 2000 and 2001. Ethanol extract of fresh nodal segments from different positions was analyzed for total soluble phenols, according to A.O.A.C. (1985) and indoles concentration as described by Larsen *et al.* (1962).

II- Explant surface disinfection and de-exudation control:

The seedlings were subjected to dark treatment (according to Wang *et al.*, 1994) by establishing a black polyethylene film as a tent over the seedlings with a suitable distance, to obtain good aeration, then explants were taken after 4 weeks. Explants were defoliated (leaving the petiole and the base of the blade) and underwent a preliminary wash with running tap water and immersed overnight in antioxidant solution consisted of 100/150 mg L^{-1} of citric/ascorbic acids. Surface sterilization was adopted under

aseptic conditions using 70% ethanol (2 min.) followed by 30% chlorox (sodium hypochlorite, NaOCl, active gradient 5.25%) with few drops of tween-20 (7 min.), as a detergent, and finally agitated at mercuric chloride solution, 0.25% (w/v) for ten minutes and three rinses in sterile distilled water was followed. Explants were basilary trimmed to a length of 10-15 mm and cultured into a medium in the dark for one week followed by rapid transfers within the same vessel and subjected to illumination, photoperiod 16/8 hours (light/ dark). These processes were adopted in order to overcome explant exudation problems. Each explant portion was marked to be simply recognized during the course of experiment and considered as a clone source.

III- Culture media and incubation conditions:

a- Culture establishment:

Two types of media, QL (Quoirin and Lepoivre, 1977) and woody plant medium WPM (Lloyd and McCown, 1980) were used and supplemented by 3% sucrose, 100 mg L^{-1} myo-inositol, 80 mg L^{-1} adenine and 1 mg L^{-1} gibberillic acid (GA_3). Media were treated with nine plant growth regulator treatments: 0.2 mg L^{-1} 3-indolebutyric acid (IBA), either alone or combined with; (0.2, 0.4, 0.6 or 0.8) mg L^{-1} N^6-benzyladenine (BA) or (0.2, 0.4, 0.6 or 0.8 mg L^{-1}) kinetin (Kin), in addition to the control treatment without any plant growth regulators supply and medium was solidified with 0.7% agar. Four explant portions from either of the aforementioned explant position were considered for each culture jar. Ten jars were employed for each treatment as replicates and incubated for six weeks involving the first week of dark treatment, under controlled conditions of temperature and light.

b- Shootlets multiplication:

Shootlets proliferated from culture establishment containing 2-3 nodes were transplanted into multiplication medium. Woody plant basal medium was modified with 1.0 mg L^{-1} GA_3, 100 mg L^{-1} myo-inositol and adenine mg L^{-1} 80. Two concentrations of sucrose (3 and 4%) were combined with the following growth regulators treatments [(0.2 mg L^{-1} IBA + 0.8 mg L^{-1} BA), (0.2 mg L^{-1} IBA + 1.0 mg L^{-1} BA) and (0.2 mg L^{-1} IBA + 1.2 mg L^{-1} BA)], in addition to control and medium was solidified with 0.7% agar. Ten jars from each multiplication treatment were considered as replicates. Cultures were incubated and three sequential sub-cultures were performed with six weeks intervals. Data were recorded in order to evaluate multiplication parameters.

c- Shootlets elongation and rooting:

Shootlets (2-3 cm height) obtained from clusters of multiplication stage were individually separated and cultured into WPM solidified basal medium which was supplemented as previously mentioned in culture establishment plus 0.3% activated charcoal for one week. Shootlets were then cultured into WPM solidified either with 0.7% agar (Difco-bacto) or 0.2% gerlite. Medium was supplemented with 3% sucrose, 1.0 mg L^{-1} GA_3, 100 mg L^{-1} myo-inositol, 80 mg L^{-1} adenine and either NAA or IBA each at similar concentrations (0.0, 0.5 and 1.0 mg L^{-1}) in combination with the two types of

the solidifying agents. Five jars were considered for each treatment as replicates and rooting criteria were recorded after 5 weeks.

All media were pH adjusted to 5.75 (± 0.05) prior to solidification and autoclaved at 121 °C and 1.2 kg cm^{-2} for 20 min. For both culture establishment and multiplication media, glass jars (ca. 200 ml) contained 30 ml of medium and for elongation stage, glass jars (ca. 400 ml) contained 40 ml medium were used and capped with PVC lids. Cultures were incubated at 25 ± 1°C under 16 hrs daily photoperiod provided by white fluorescent tubes (Phillips) 40 µE cm^{-2} s^{-1} light intensity and 40% relative humidity (RH).

Experiments were repeated twice at least and data were subjected to analysis of variance according to Steel and Torrie (1980) assuming a complete randomized design. Mean separation was made using least significant difference (L.S.D.) at 5% level of significance. Percentage data were subjected to an arcsin transformation.

RESULTS AND DISCUSSION

Dark treatment of seedlings brought about obvious overcoming for the explant production of inhibitory phenolic exudates, consequently, giving rise to culture establishment characters. Supporting results were obtained when leaves of *Eucalyptus grandis* were soaked in the light, there were more total phenols compared to soaking in the dark (Durand-Cresswell *et al.*, 1982). Moreover, Yu and Meredith (1986) found that portions of stock plants of grape that were shaded, survived better *in vitro* and had significantly lower levels of total phenols than those portions taken from stock plants received full sunlight.

1. Indoles and phenols compounds

The concentration of indoles and phenols was highly influenced by the explant position on *Quercus robur* L. seedling's branch. The greatest indoles concentration (2.06 mg/g) was observed in the basal nodes, while the least value (1.64 mg/g) was recorded in the shoot tip as represented in Fig. (1). Conversely, the greatest value of phenols (2.53 mg/g) was obtained in shoot tip and the least value (1.90 mg/g) was recorded in the basal nodes. Moreover, the ratio of indoles/phenols exhibited similar trend as obtained in indoles concentration in the various explants (1.10, 0.80 and 0.65) in basal nodes, medial nodes and shoot tip, respectively. This indicates that indoles concentration along the branch axis is declining from the base to the tip of the branch and *vice versa* in concern with phenols concentration. These results may be due to phenolic compounds, such as cinnamic acid, inhibit auxin activity, hence, decreasing indoles concentration by increasing phenols could be explained according to this finding. These results coincide with those suggested by Kramer and Kozlowski (1979) who also indicated that favourable balance of endogenous growth promoters over the inhibitors plays a central role in regulating the vegetative growth of woody plants. Moreover, Hu and Wang (1983) suggested that oxidized polyphenolic compounds inhibit enzyme activity and may result in lethal browning of the explants.

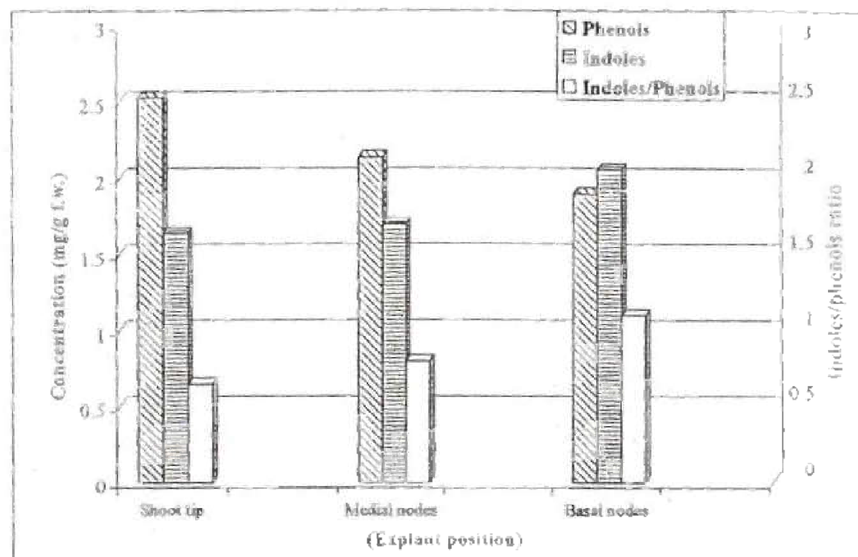

Fig. (1): Effect of explant position as located on *Quercus robur* L. seedling's branch on phenols, indoles concentration (mg/g f.w.) and indoles/phenols ratio.

2. Culture establishment

2.1. Explant survival

Data represented in Table (1) reveal that explant survival significantly influenced by explant position and its interaction with plant growth regulators when cultured on WPM, while significantly influenced by explant position only when cultured on QL medium. Plant growth regulators were not a controlling factor on survival of explants neither when cultured on WPM nor on QL media. The highest values of explant survival were significantly observed (66.9 and 27.89%) with the basal nodes cultured on WPM and QL media, respectively. On the other hand, the lowest values (47.62% and 7.05%) were significantly recorded when shoot tip explant was cultured on WPM and QL media, respectively. Moreover, the highest interaction value between explant position and growth regulators significantly was (88.18%) observed on WPM which was induced when the shoot tip cultured on medium supplemented with 0.2 mg L^{-1} IBA + 0.4 mg L^{-1} Kin and both of medial and basal nodes were cultured on medium with 0.2 mg L^{-1} IBA + 0.8 mg L^{-1} BA. However, medial nodes explant when cultured on WPM supplemented with 0.2 mg L^{-1} IBA + 0.4 mg L^{-1} Kin, no result was obtained.

2.2. Shootlets number

Shootlets number per explant is highly influenced by explant position (E.P.) cultured on both media (WPM and QL), and by plant growth regulators (PGR) modified to WPM only, as observed in Table (1) and Fig. (2 a).

Table (1): Effect of explant position and plant growth regulators modified to WPM and QL media on explant survival%, shootlets number/explant and number of leaves/shootlet during culture establishment of *Quercus robur* L.

Growth characters	Explant survival (%)				Shootlets number/explant				Leaves number/shootlet			
Explant position (E.P.) / Plant growth regulators (P.G.R.) mg L^{-1}	Shoot tip	Medial nodes	Basal nodes	Mean P.G.R.	Shoot tip	Medial nodes	Basal nodes	Mean P.G.R.	Shoot tip	Medial nodes	Basal nodes	Mean P.G.R.
WPM-medium												
Control	52.91	44.63	79.54	59.03	0.60	0.40	0.70	0.57	1.60	2.20	5.73	3.18
IBA (0.2)	70.54	16.05	52.22	46.27	0.80	0.40	0.37	0.52	2.20	2.70	6.80	3.90
IBA (0.2) + BA (0.2)	17.64	62.78	51.32	43.91	0.20	1.20	0.80	0.73	0.40	0.83	5.00	2.08
IBA (0.2) + BA (0.4)	35.27	44.27	70.54	50.03	1.00	2.00	0.20	1.07	1.04	1.47	1.00	1.17
IBA (0.2) + BA (0.6)	17.64	44.63	79.54	47.27	0.00	3.20	2.00	1.73	0.00	3.39	4.99	2.79
IBA (0.2) + BA (0.8)	70.54	88.18	88.18	82.30	0.80	3.40	1.11	1.77	0.70	4.23	3.00	2.64
IBA (0.2) + Kin (0.2)	52.91	72.86	75.76	67.18	0.40	0.10	0.90	0.47	2.40	2.53	8.20	4.38
IBA (0.2) + Kin (0.4)	88.18	0.00	55.22	47.80	0.20	0.00	0.60	0.27	0.00	0.00	4.20	1.40
IBA (0.2) + Kin (0.6)	35.27	63.86	54.72	51.28	0.20	0.30	0.20	0.23	0.40	2.80	5.20	2.80
IBA (0.2) + Kin (0.8)	35.27	70.54	61.91	55.91	0.40	0.40	0.33	0.38	1.00	3.30	2.90	2.40
Mean (E.P.)	47.63	50.78	66.90		0.46	1.14	0.72		0.97	2.35	4.70	
L.S.D. (5%)												
E.P.	13.81				0.547				1.58			
P.G.R.	N.S.				0.999				N.S.			
E.P.×P.G.R.	23.93				N.S.				N.S.			
QL-medium												
Control	0.00	0.00	18.00	6.00	0.00	0.00	0.21	0.07	0.00	0.00	1.10	0.37
IBA (0.2)	0.00	26.64	26.64	17.76	0.00	0.30	0.30	0.20	0.00	1.00	1.90	0.97
IBA (0.2) + BA (0.2)	0.00	18.00	24.68	14.23	0.00	0.70	0.47	0.39	0.00	1.23	6.00	2.41
IBA (0.2) + BA (0.4)	0.00	35.27	17.64	17.64	0.00	0.90	0.10	0.33	0.00	2.82	0.00	0.94
IBA (0.2) + BA (0.6)	17.64	26.64	42.32	28.87	0.20	0.20	2.00	0.80	0.00	0.00	2.50	0.83
IBA (0.2) + BA (0.8)	0.00	0.00	44.63	14.88	0.00	0.00	0.56	0.18	0.00	0.00	2.20	0.73
IBA (0.2) + Kin (0.2)	0.00	0.00	18.00	6.00	0.00	0.00	0.30	0.10	0.00	0.80	2.60	1.13
IBA (0.2) + Kin (0.4)	35.27	26.64	44.27	35.39	0.40	0.30	0.50	0.40	3.20	2.40	3.00	2.87
IBA (0.2) + Kin (0.6)	17.64	44.27	25.05	28.99	0.20	0.20	0.33	0.24	2.00	2.30	2.80	2.37
IBA (0.2) + Kin (0.8)	0.00	9.00	17.64	8.88	0.00	0.10	0.20	0.10	0.00	0.60	1.00	0.53
Mean (E.P.)	7.05	18.64	27.89		0.08	0.27	0.50		0.52	1.12	2.31	
L.S.D. (5%)												
E.P.	12.23				0.305				1.13			
P.G.R.	N.S.				N.S.				N.S.			
E.P.×P.G.R.	N.S.				N.S.				N.S.			

However, the interaction between both (E.P. and P.G.R.) exerted no significant influence on shootlets number/explant. The basal nodes significantly surpassed the other two explant positions by inducing the highest shootlets number per explant (0.497) when cultured on QL medium, however, the medial nodes exceeded the other two explants when cultured on WPM, giving rise to shootlets number to (1.14 shoots). Concerning the affect of plant growth regulators, the modification of 0.2 mg L^{-1} IBA + 0.8 mg L^{-1} BA to WPM induced the highest shootlets number per explant (1.77) which gradually decreased by increasing BA concentration and by increasing Kin

concentration as well. The lowest shootlets number was (0.23) by applying 0.2 mg L^{-1} IBA + 0.6 mg L^{-1} BA to the medium. Generally, the highest shootlets number was observed (3.4) by culturing medial nodes explant on WPM supplemented with 0.2 mg L^{-1} IBA + 0.8 mg L^{-1} BA, however, no significant difference was determined due to the interaction effect.

2.3. Leaves number

Data illustrated in Table (1) exhibit the significant effect of explant type on leaves number per shootlet. The highest leaves number (4.70) per shootlet was significantly produced due to culturing the basal node on WPM exceeding those on QL medium (2.31). However, when the same explant was cultured on WPM supplemented with 0.2 mg L^{-1} IBA + 0.2 mg L^{-1} Kin and on QL medium modified with 0.2 mg L^{-1} IBA + 0.2 mg L^{-1} BA, leaves number was increased to (8.20 and 6.0), respectively. No significant difference between means was obtained neither due to growth regulators treatments nor its interaction with the explant type.

In general, WPM surpassed QL medium, as well, the explant position significantly influenced all culture establishment characters with especial concern to the basal nodes, except for shootlet number per explant, since the medial nodes augmented the highest shootlet number significantly on WPM.

The enhancing effect of explant position to growth characters might be interpreted according to the aforementioned analysis of phenols and indoles. The basal nodes contained the lowest phenols, as growth inhibitors and the highest indoles, as growth promoters as compared to the other explant positions, resulting in lower inhibition of auxin activity exerted by phenols. It was observed by Hu and Wang (1983) and Preece and Compton (1991) that when plants were injured, phenolic compound in the vacuole are mixed with the contents of plastids then the dark pigmentation associated with the oxidized polyphenolic compounds appears. Moreover, Stenlid (1976) and Hanafy Ahmed *et al.* (2002 a) assumed that phenols may have indirect effects on several physiological processes, such as inhibiting enzyme activity, antagonizing plant hormones biosynthesis and inhibiting ions absorption through alteration in the permeability of the membrane resulting in decreasing ions absorption or even loss of previously absorbed ions and/or other metabolites which affect the polar transport of auxins. It was suggested by Kramer and Kozlowski (1979) that auxins induce cambial activity and promote fibers and vessels elongation, however, cambial response to auxins often is not precisely correlated with the amount of auxin present. Confirming results were reported by San Jose *et al.* (1990) on sessile oak, Al Saqri and Alderson (1996) on *Rosa centifolia* and Puddephat *et al.* (1997) on *Quercus robur*.

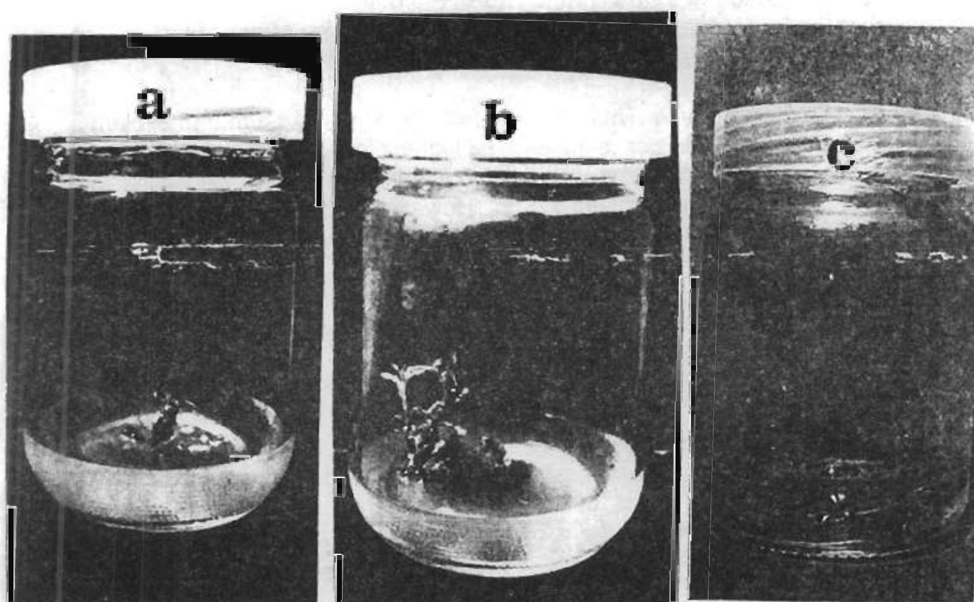

Fig. (2): a- Shootlets proliferation on WPM supplemented with 0.2 mg L^{-1} IBA + 0.8 mg L^{-1} BA
b- Shootlets multiplication on WPM supplemented with 0.2 mg L^{-1} IBA + 1.2 mg L^{-1} BA
c- Root formation on WPM supplemented with 0.5 mg L^{-1} IBA and solidified by 0.2% gerlite

3. Shootlets multiplication

Data illustrated in Table (2) reveal that sucrose concentration had an effective role controlling the growth parameters of shootlets multiplication followed by the effect of growth regulators, however, the interaction between both factors controlled shootlets number only.

The highest shootlets number per explant significantly took place (19.23) due to modifying the medium by (3%) sucrose, exceeding shootlets number obtained due to applying (4%) sucrose to the medium (2.45).

Moreover, the greatest concentration of BA (1.2) mg L^{-1} combined with 0.2 mg L^{-1} IBA improved shootlets number significantly (32.0 shootlets) compared to those obtained on control (0.2 shootlets). it is observed that by increasing BA concentration shootlets number increased. Meantime, providing the medium by 3% sucrose and 0.2 mg L^{-1} IBA + 1.2 mg L^{-1} BA increased shootlets number significantly to (60.0) shootlets per explant compared to control (0.27 shootlets) with more than two hundred its value.

Table (2): Effect of sucrose concentration and plant growth regulators modified to WPM on the efficiency of shootlets multiplication characters after three subcultures.

Plant growth regulators (P.G.R.) mg L^{-1}	Shootlets number			Shootlet height (cm)			Leaves number/shootlet		
	Sucrose (3.0%)	Sucrose (4.0%)	Mean P.G.R.	Sucrose (3.0%)	Sucrose (4.0%)	Mean P.G.R.	Sucrose (3.0%)	Sucrose (4.0%)	Mean P.G.R.
Control	0.27	0.13	0.20	3.33	1.33	2.33	3.00	1.17	2.09
IBA (0.2) + BA (0.8)	5.00	2.33	3.67	2.50	1.00	1.75	4.33	1.33	2.83
IBA (0.2) + BA (1.0)	11.66	3.33	7.50	2.17	1.33	1.75	5.00	3.00	4.00
IBA (0.2) + BA (1.2)	60.00	4.00	32.00	1.50	1.13	1.32	6.33	4.00	5.17
Mean sucrose (S)	19.23	2.45		2.38	1.20		4.67	2.38	
L.S.D. (5%)									
P.G.R.		3.00			N.S.			1.50	
S		2.12			0.79			1.06	
P.G.R.×S.		4.24			N.S.			N.S.	

Shootlets height was significantly influenced by sucrose concentration only. The maximum shootlet height (2.38 cm) was obtained due to applying 3% sucrose, compared to the value induced due to providing higher sucrose concentration (1.2 cm). However, control treatment produced the longest shootlet (3.33 cm) by modifying the medium with 3% sucrose, but with no significant difference between mean values of interaction.

Concerning the effect of sucrose concentration on leaves number per shootlet, it is observed that 3% sucrose generated the highest leaves number significantly (4.67/shootlet) compared to the higher concentration of sucrose (2.38 leaves/shootlet). Meanwhile, the highest BA concentration (1.2) mg L^{-1} combined with 0.2 mg L^{-1} IBA induced the highest leaves number per shootlet, significantly (5.17) compared to control which produced the least leaves number per shootlet (2.08). The interaction effect between 3% sucrose and 0.2 mg L^{-1} IBA + 1.2 mg L^{-1} BA generated the highest leaves number per shootlet (6.33), however with no significant difference between means.

The obtained results indicate the promotive effect of sucrose concentration (3%) as well as the combined growth regulators 0.2 mg L^{-1} IBA + 1.2 mg L^{-1} BA on producing the highest shootlets number and highest leaves number per explant. However, the longest shootlet was induced on control treatment supplemented with 3% sucrose. These results might be attributed to the effect of the cytokinin 1.2 mg L^{-1} BA combined with its sixth value of auxin 0.2 mg L^{-1} IBA in stimulating cell division and both of formation and growth of axillary shoots inducing higher nodes number involving higher number of leaves on the expense of cell elongation, hence, producing shorter shoots. Cytokinins have been shown to activate RNA synthesis and to stimulate protein synthesis and enzyme activity (Kulaeva, 1980). These results are in harmony with those obtained by Peng *et al.* (1997) on *Philodendron erubescence*, Zayed (2000) on *Spathiphyllum wallisii* and

Hussein (2002) on three *Aglaonema species*. Concerning the effect of sucrose (3.0%) concentration on growth characters, it could be due to its effect on regulating the pathway of metabolites enhancing cell division and differentiation. Supporting results were reported by Shoyama *et al.* (1992) on *Quercus acutissima* and Cheng *et al.* (1995) on *Eucommia ulmoides*. However, opposing results were obtained by Maene and Debergh (1985) on *Philodendron erubescens* K. Koch and *Cordyline terminalis* L. Kunth and Cheng *et al.* (1992) on *Eucalyptus sideroxylon*. These contrasting results may be attributed to genera and species difference effect.

4. Rooting ability of shootlets

The ability of *in vitro* shootlets to induce rooting is significantly influenced by the type of the solidifying agent as well as type and concentration of the auxin applied to WPM, as revealed in Table (3).

Data illustrate that gerlite (0.2%) surpassed agar (0.7%), as solidifying agents, in inducing higher rooting ability (30%) compared to that induced by agar (6%). Meanwhile, IBA exceeded NAA, significantly in giving rise to rooting into 45%. Moreover, the highest overall rooting (60%) took place due to applying IBA at either concentration (0.5 or 1.0) mg L^{-1} to WPM medium solidified by gerlite.

On the other hand, each of root length and roots number (1.53 cm and 1.50), respectively, were significantly higher due to applying IBA to the medium rather than with NAA, with especial regard to the low concentration of IBA (0.5) mgL^{-1}.

Table (3): Effect of solidifying agent type (agar and gerlite) and plant growth regulators modified to WPM on rooting criteria during shootlets rooting of *Quercus robur* L.

Plant growth regulators (P.G.R.) mg L^{-1}	Rooting (%)			Root length (cm)			Roots number/shootlet		
	Agar (0.7%)	Gerlite (0.2%)	Mean P.G.R.	Agar (0.7%)	Gerlite (0.2%)	Mean P.G.R.	Agar (0.7%)	Gerlite (0.2%)	Mean P.G.R.
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
IBA (0.5)	30.00	60.00	45.00	1.67	1.40	1.54	0.67	2.33	1.50
IBA (1.0)	0.00	60.00	30.00	0.00	0.27	0.14	0.00	0.67	0.33
NAA (0.5)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
NAA (1.0)	0.00	30.00	15.00	0.00	0.13	0.07	0.00	0.13	0.07
Mean solidifying agent (S.A.)	6.00	30.00		0.33	0.36		0.13	0.63	
L.S.D. (5%)									
P.G.R.	31.29			0.63			0.39		
S.A.	19.97			N.S.			0.25		
P.G.R.×S.A.	N.S.			N.S.			0.55		

Moreover, gerlite remarkably brought about the greatest root number per shoot (0.63) compared to those obtained by agar (0.13). The combined interaction between the solidifying agent and plant growth regulators generated the highest observed roots number per shoot (2.33) as a result to the combined supplement of gerlite and 0.5 mg L^{-1} IBA to WPM. Nevertheless, neither the solidifying agent nor the combined interaction significantly influenced root length. Besides, no root formation was observed with WP basal medium (control).

The obtained results indicate that gerlite is an alternative gelling agent which forms clear gel and is free of contaminating compounds that may counteract the uptake of some medium components. Zimmerman and Robacker (1988) on cotton and Ibrahim (1994) on *Cordyline terminalis* obtained similar results.

On the other hand, the preponderance of IBA might be due to its considerable stability for decomposition, as well, it can be related to species-specific concerns. These results are in harmony with those obtained by Shoyama *et al.* (1992) and Puddephat *et al.* (1999) on *Quercus robur* and Hussein (2002) on *Aglaonema spp.*

The practical implication of this work is that manipulating the mother plant followed by handling the sterilized explants should be adopted in order to overcome explant exudation problems then the following work will become easier.

It could be concluded that micropropagation from juvenile material in standard tissue culture conditions became possible, even when stubborn obstacles, such as phenol exudates appear. Studying the explant position on the mother plant plays a significant role in culture establishment success. The type of exogenously applied growth regulators, as well as investigating the appropriate balance between them, sucrose concentration, the gelling agent type and concentration and the other nutritional and physical factors should be considered for optimizing the output of the work.

This protocol should eventually find applicability with mature trees, using more appropriate modifications, to avoid the excessive phenols exudates as the problem is enormous. Further work should be adopted, as well, on optimizing the acclimatization of *ex vitro* produced plantlets followed by successful transplantation to the field.

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كفاءة البلوط .*Quercus robur* L علي الانتاج المكثف من النبتـــات مــن خـــلال تكنيك زراعة الانسجة و العوامل المؤثرة علي نجاحه

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كان الهدف من هذا العمل البحثي هو الحصول علي إنتاج مكثف من نباتات البلوط باعتبارها مـــن الاشجار الواعدة. كانت هناك مشكلة رئيسية تعوق العمل منذ بدايته ألا و هـــي الإرتشـاحات الفينوليـــة مـــن النباتات و التي أمكن التغلب عليها بمعاملة النباتات الام بنظام اظلامي و اتبعه خطوات متتالية بمعاملة المعـزلات النباتية المتحصل عليها. كانت هناك تأثيرات واضحة لوضع العزلة النباتية علي فرع النبات الام و نوع البيئـة المستخدمة و تركيز السكروز المستخدم و المادة المضافة لتصلب البيئة (الأجـــار و الجـيرلايت) و كذلـك منظمات النمو المضافة للبيئة (نوعها و تركيزها) علي النتائج المتحصل عليها. فقـــد كـــان لزراعـــة العقـد الوسطية علي بيئة النباتات الخشبية WPM مضافا اليها كل من البنزايل ادينين (٠,٨ مجم / لتر) + حمـــض اندول البيوتريك (٠,٢ مجم / لتر) تأثير محسن علي مواصفات مرحلة انشاء المزروعة و ذلك بالتفوق علـــي استخدام العزلات السفلي و القمة النباتية و كذلك علي استخدام بيئـــة QL. كمـــا أدت إضافـــة البنزيـــن (١,٢ مجم/لتر) + حمض اندول البيوتريك (٠,٢ مجم / لتر) + ٣٠ جم سكروز إلي بيئة النباتات الخشبية WPM إلـــى الإنتاج المكثف من تضاعف النباتات (٦٠ نبتة لكل عزلة نباتية). كما تم الحصول علي تجذير جيد للنباتـــات بزراعتها علي بيئة WPM مضافا اليها حمض اندول البيوتريك بتركيز (٠,٥ مجم / لتر) كمـــا أن إضافـــة الجيرلايت بتركيز (٠,٢٪) تفوقت علي اضافة الأجار (٠,٧٪) في إعطـــاء نتـــائج افضـــل للتجذيـــر حيـــث استخدمت كمواد مساعدة لتصلب البيئة.